6-Azauridine Triacetate Induced Hyper β-Alaninemia and Its Decrease by Administration of Pyridoxine

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Summary The effect of pyridoxine on 6-azauridine triacetate (6-AzUrd-TA) induced hyper β-alaninemia was studied in New Zealand albino rabbits in three experiments. In each of the three experiments the animals were administered by gavage: Group 1 (Control), drinking water; Group 2, 6-AzUrd-TA; and Group 3, 6-AzUrd-TA with pyridoxine. While no β-alanine was found in the control group or in pretreatment samples of the 6-AzUrd-TA and 6-AzUrd-TA + pyridoxine treated animals, high concentrations of this amino acid (191.0 ± 91.6, 220.2 ± 116.3, 103.2 ± 64.4 nmol/ml) were found on the fourth and seventh days of 6-AzUrd-TA treatment with daily doses of 1.0 g/kg and 0.5 g/kg B.W. respectively. The drug induced hyper β-alaninemia was significantly (p ≤ 0.05) reduced in all three experiments by simultaneous pyridoxine administration in daily doses of 50 mg/kg B.W. These results indicate, that daily repeated oral administration of 6-AzUrd-TA causes elevation of serum β-alanine, which can be partially prevented by oral administration of pyridoxine. They also indirectly support the hypothesis, that 6-AzUrd-TA induced hyper β-alaninemia is at least partially caused by the inhibition of β-alanine degrading enzymes, that use pyridoxal phosphate as a coenzyme. Direct measurement of the enzyme activity is planned in our future studies.

Key Words 6-azauridine triacetate, β-alanine, pyridoxine, pyridoxal phosphate

6-Azauridine triacetate (6-AzUrd-TA), generically termed Azaribine, is an anticancer drug with clinical activity in treatment of psoriasis (1, 2), mycosis fungoides (1, 3), trophoblastic malignancies (4), and polycythemia vera (5). After oral administration, 6-AzUrd-TA is subjected to nearly complete deacetylation either during or after its absorption from the intestine to yield 6-azauridine (6-AzUrd),

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that after intracellular phosphorylation to 6-AzUrd 5'-monophosphate inhibits orotidine-5'-monophosphate decarboxylase, the terminal and rate limiting enzyme in the de novo biosynthesis of uridine-5'-monophosphate (6, 7). Additional biochemical changes caused by this drug involve those resulting from its interference with pyridoxal phosphate coenzyme (8, 9) and consequently with metabolism of various amino acids (10–13). This paper presents results on hyper β-alaninemia following administration of 6-AzUrd-TA in New Zealand albino rabbits and a significant decrease of this biochemical change by administration of pyridoxine.

METHODS

New Zealand albino rabbits of both sexes, weighing 1.5 to 4.5 kg, were used in three experiments. The animals were accommodated in the Animal Resource Facility, University of New Mexico School of Medicine, 2 weeks before the start of the experiment. They were housed separately in metal cages and were kept on a standard diet of Wayne’s Rabbit Ration (Allied Mills, Inc., Chicago, IL).

In each of the three experiments the animals were divided equally into three groups: 1) The control group administered water twice a day by gastric gavage, 2) the group administered 6-AzUrd-TA twice a day by gastric gavage, and 3) the group administered 6-AzUrd-TA and pyridoxine twice a day by gastric gavage. In the first two experiments, 6-AzUrd-TA was administered in the daily doses 1 g/kg body weight, divided into two equal portions of which the first was given six hours before the second half, in the third experiment the daily dose of 6-AzUrd-TA was 0.5 g/kg body weight, again administered in the twice a day schedule corresponding to that one in the two proceeding experiments. Pyridoxine in 10% solution, with daily doses of 50 mg/kg divided into two equal portions, was administered orally by gavage at the same time intervals with azaribine. Blood samples were taken before treatment and after the administration of each second daily portion of the drug on the fourth or seventh day. The animals were fasting for at least 2 h before each blood sample was drawn. Trichloracetic acid supernates of serum were prepared and analyzed for β-alanine using standard techniques for analysis of physiologic fluids (14) on a Beckman 120 C amino acid analyzer equipped for high sensitivity. Student’s paired t-test was used for the statistical analysis of the changes in the concentration of β-alanine in the serum after oral administration of azaribine, in comparison to the pre-treatment values. The differences in serum β-alanine concentrations between the 6-AzUrd-TA and 6-AzUrd-TA plus pyridoxine treated groups of rabbits were statistically evaluated using Student’s t-test.

RESULTS

The results of all three experiments are summarized in Table 1. While no or only trace (≤2 nmol/ml) amounts of β-alanine were detected in the pretreatment serum samples of all three groups of animals and all serum samples of controls, high

Table 1. Serum β-alanine (nmol/ml) in New Zealand Albino rabbits.

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1ª</th>
<th>Experiment 2ª</th>
<th>Experiment 3ª</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=5)</td>
<td>(n=8)</td>
</tr>
<tr>
<td>Before</td>
<td>191.9 ± 91.6</td>
<td>220.2 ± 116.3</td>
<td>103.2 ± 64.4</td>
</tr>
<tr>
<td>4th day</td>
<td>83.0 ± 39.6</td>
<td>84.6 ± 46.6</td>
<td>43.5 ± 22.4</td>
</tr>
<tr>
<td>6-AzUrd-TA</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>6-AzUrd-TA + Pyridoxine</td>
<td>191.9 ± 91.6</td>
<td>220.2 ± 116.3</td>
<td>103.2 ± 64.4</td>
</tr>
</tbody>
</table>
| * not detectable = less than 2 nmol/ml.

levels of β-alanine were found in all 6-AzUrd-TA treated rabbits on the fourth and seventh days of 6-AzUrd-TA treatment. The changes in serum β-alanine levels are statistically significant at the p ≤ 0.05 level, in comparison to the pretreatment values.

In all three experiments, hyper β-alaninemia was significantly (p ≤ 0.05) decreased in 6-azUrd-TA treated animals who were simultaneously given pyridoxine. As shown in Table 1, serum β-alanine levels in the 6-AzUrd-TA + pyridoxine treated groups of animals reached only less than 50% of serum β-alanine levels reached in animals treated with 6-AzUrd-TA treatment alone.

DISCUSSION

The results presented in this paper indicate that orally administered 6-AzUrd-TA causes in rabbits a significant increase of β-alanine in the serum and confirm our previous findings from experimental animals and human studies (10-13). Our results are also in agreement with our clinical observations that β-alanine urinary excretion is quantitatively dependent on the 6-AzUrd-TA administered dose (12, 13). In this study, the serum β-alanine levels after administration of repeated doses of 0.5 g/kg body weight of 6-AzUrd-TA reached about half of β-alanine concentrations observed after same duration of treatment (7 days) in the rabbits treated with the double (1 g/kg/day) dose of this drug.

β-Alanine is a component of anserine, carnosine, and coenzyme A and has been suggested to function as an inhibitory neurotransmitter (15). The mechanism of hyper β-alaninemia induced by 6-AzUrd-TA has not been fully elucidated. It could be explained by an increased β-alanine biosynthesis, or a decreased β-alanine catabolism or by combination of both factors. An increased β-alanine biosynthesis could arise from carbamyl aspartic acid formed in increased amounts after 6-AzUrd-TA administration, and its cleavage by carbamyl-l-aspartic decarboxylase to carbamyl β-alanine, which is further catabolized to β-alanine (16, 17). Also a
surprisingly increased production of uridine-5-phosphate reported in 6-AzUrd treated patients (18) in spite of orotidylic acid decarboxylase inhibition could be a possible source of \( \beta \)-alanine formed from uracil via ureidopropionic acid (10, 13, 17). The hypothesis that 6-AzUrd-TA induced hyper \( \beta \)-alaninemia is caused by decreased degradation of \( \beta \)-alanine is supported by results of recently published studies revealing direct and indirect evidence for inhibition of enzymes involved in catabolism of this amino acid. In vitro and in vivo inhibition of \( \beta \)-alanine-2-oxoglutarate aminotransferase activity by 6-azauracil with consequent increase of \( \beta \)-alanine in rat liver tissue has been recently reported (19, 20). Also our results (8, 9) on the significant decrease of pyridoxal phosphate levels in the serum of rabbits treated with 6-AzUrd-TA suggest the inhibition of enzymes controlling \( \beta \)-alanine degradation, using pyridoxal phosphate as a coenzyme.

The results presented in this paper revealing that administration of pyridoxine significantly decreases 6-AzUrd-TA induced hyper \( \beta \)-alaninemia also support the hypothesis that the elevation of this amino acid is at least partially caused by the inhibition of enzymes using pyridoxal phosphate as a coenzyme. However, since only a significant decrease of \( \beta \)-alaninemia and not its complete prevention is achieved with pyridoxine, both increased biosynthesis as well as decreased catabolism of \( \beta \)-alanine may be responsible for its elevation in the serum after repeated administration of 6-AzUrd-TA.

REFERENCES


